Visual Pigments. III. Determination and Interpretation of the Fluorescence Quantum Yields of Retinals, Schiff Bases, and Protonated Schiff Bases[1]

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Abstract: The fluorescence quantum yields (ϕ_F) of *all-trans*-, 9-*cis*-, and 13-*cis*-retinal, their corresponding *n*-butylamine Schiff bases, the methyl Schiff base of *all-trans*-retinal, and the protonated methyl and *n*-butyl Schiff bases of *all-trans*-retinal have been determined at 77°K. No fluorescence is observed from any of the foregoing molecules at room temperature, $\phi_F < 0.001$. The dependence of the quantum yield upon the excitation wavelength was calculated from the fluorescence excitation spectrum for the isomeric retinals and Schiff bases. While ϕ_F showed only a small dependence upon the exciting wavelength for the Schiff bases, there was a large variation when exciting into the long wavelength absorption region (400–440 nm) in the retinals. Utilizing the known data on ϕ_{ISC} and ϕ_{PI} an analysis of the fate of the absorbed quanta is carried out for several of the molecules. The differences in the spectroscopic behavior of retinals and Schiff bases are interpreted primarily on the basis of the existence of a $^1(n,\pi^*)$ state at energies comparable to the two lowest energy $^1(\pi,\pi^*)$ states in the retinals.

Previously, we have examined the emission spectral properties of the all-trans, 9-cis, 11-cis, and 13-cis isomers of retinal.[1,2] Since the linkage to retinal in rhodopsin has been shown to be a Schiff base linkage *via* a primary amino group of lysine,[3] we have extended this study to include the *n*-butylamine Schiff bases of the retinal isomers. We also have investigated the methylamine Schiff base of *all-trans*-retinal, as well as the protonated methyl and *n*-butyl trans Schiff bases. In addition, we have reinvestigated *all-trans*-retinal and have quantitative data on the 9-*cis*- and 13-*cis*-retinals.

Fluorescence has been reported from the tryptophans[4] of rhodopsin as well as rhodopsin[5] itself. A weak emission, $\phi_F = 0.005$, centered around 575 nm was found at both 276 and 77°K;[5] however, more recent attempts to observe this emission from rhodopsin were unsuccessful.[6] Several of the intermediates in the bleaching process of rhodopsin[7] have been observed to fluoresce.[8] Although no fluorescence was observed from prelumirhodopsin, emission was observed from lumirhodopsin and both metarhodopsin I and II at 77°K. Emission from *N*-retinylidene opsin[8] resembled that of *all-trans*-retinal.[1,2]

Thompson[9] has reported emission from the isomeric retinols at room temperature and a series of compounds containing the retinyl chromophore, including the methyl Schiff base of *all-trans*-retinal at 77°K. A fluorescence has been detected for the *n*-butyl Schiff base of *all-trans*-retinal.[10] The emission maximized at 486 nm at 77°K in both 3MP and EPA. When a trace of acetic acid was added to either solvent, a red shift was observed in the fluorescence maximum to 540 nm.[10]

Experimental Section

all-trans-, 9-*cis*-, and 13-*cis*-retinal were purchased from Sigma Chemical Co., while 11-*cis*-retinal was a generous gift of the Hoffmann-La Roche Chemical Co. All compounds were stored in the dark under vacuum at 0°. There was no further purification prior to usage.

n-Butylamine was fractionally distilled and stored at ambient temperatures over a 4A molecular sieve. Preparation of the *n*-butylamine Schiff base of *all-trans*-retinal was similar to that previously reported.[10,11] A retinal sample was dissolved in a small amount of methanol and cooled in a Dry Ice bath; then a molar excess of *n*-butylamine was added. The mixture was allowed to react at 0° over a 3A molecular sieve. After conversion of the aldehyde to the imine, as indicated by a change in the uv absorption maxima, the mixture was filtered and evaporated to dryness using a vacuum pump. The 9-cis, 11-cis, and 13-cis Schiff bases were prepared *in situ* by addition of an equimolar amount of *n*-butylamine to a 10^{-4} *M* solution of the retinal in 3-methylpentane. Retinal and Schiff base spectral data was obtained from $\sim 10^{-4}$ *M* solution.

Protonation of the methyl or *n*-butyl trans Schiff bases was done at room temperature using anhydrous HCl gas that was prepared *in situ* by bubbling the gas directly into the sample contained in a 2-mm cell. The mixture was then cooled to 77°K in liquid nitrogen.

The solvent for all experiments was 3-methylpentane (3MP), which was purchased from Phillips petroleum as 99+ mol % pure. It was subsequently refluxed and distilled from dri-Na and passed through an 18-in. column of silver nitrate-alumina.[12] Flat faced 2-mm cells were used in conjunction with flat-faced liquid nitrogen dewars. All optical quartz was of the Suprasil II variety.

Absorbance and per cent transmission spectra were recorded at both room (298°K) and low (77°K) temperatures using a Cary Model 15 spectrophotometer.

Emission spectra were recorded with an apparatus that included a 1000 W dc Xenon lamp with a Bausch & Lomb 1/4M grating monochromator to excite at selected wavelengths. The emission was monitored at an angle of 20–30° from the incident beam with a scanning Aminco 1/4M grating monochromator. The analyzing monochromator was equipped with a 9558 QA EMI photomultiplier tube. Spectra were recorded on a X-Y recorder. A simple modification of the apparatus allowed recording of the excitation spectrum by driving the Bausch & Lomb monochromator.

(1) Paper II: R. S. Becker, K. Inuzuka, J. King, and D. E. Balke, *J. Amer. Chem. Soc.*, **93**, 43 (1971).
(2) D. E. Balke and R. S. Becker, *J. Amer. Chem. Soc.*, **89**, 5061 (1967).
(3) W. J. DeGrip, S. L. Bonting, and F. J. M. Daemen, *Biochim. Biophys. Acta*, **303**, 189 (1973).
(4) T. G. Ebrey, *Photochem. Photobiol.*, **15**, 585 (1972).
(5) A. V. Guzzo and G. L. Pool, *Science*, **159**, 312 (1968).
(6) G. E. Busch, M. L. Applebury, A. A. Lamola, and P. M. Rentzepis, *Proc. Nat. Acad. Sci. U. S.*, **69**, 2802 (1972).
(7) E. W. Abrahamson and S. E. Ostroy, *Progr. Biophys. Mol. Biol.*, **17**, 179 (1967).
(8) A. V. Guzzo and G. L. Pool, *Photochem. Photobiol.*, **9**, 565 (1969).
(9) A. J. Thompson, *J. Chem. Phys.*, **51**, 5106 (1969).
(10) C. S. Irving and P. A. Leermakers, *Photochem. Photobiol.*, **1**, 665 (1968).
(11) W. H. Waddell and R. S. Becker, *J. Amer. Chem. Soc.*, **93**, 3788 (1971).
(12) E. C. Murray and R. N. Keller, *J. Org. Chem.*, **34**, 2234 (1969).

Table I. Quantum Yields of Retinals and Their *n*-Butyl Schiff Bases

Compd	Absorption[a] max, nm	Emission[a] max, nm	Fluorescence[b] quantum yield ϕ_F	Exciting[c] wavelength, nm
all-trans-Retinal	384	510	0.045^d	440
9-*cis*-Retinal	381	525	0.03_5	430
13-*cis*-Retinal	381	505	0.12	440
all-trans-n-Butyl Schiff base[e]	364	490	0.07	360
9-*cis-n*-Butyl Schiff base	365	525	0.02	350
13-*cis-n*-Butyl Schiff base	362	490	0.05	350
all-trans-n-Butyl SB + HCl[e]	460	680	0.005	475
all-trans-n-Butyl SB + HCl	542	~685	0.03	525

[a] All spectra recorded at 77°K in 3MP at concentrations of $\sim 10^{-4}$ *M*. [b] Relative to 9,10-diphenylanthracene, $\phi_F = 1.0$. [c] Exciting wavelength where ϕ_F has a maximum value. [d] See text and ref 20. [e] The methyl and protonated methyl Schiff bases have quantum yields and band maxima similar to the *n*-butyl and protonated *n*-butyl Schiff bases of *all-trans*-retinal, respectively.

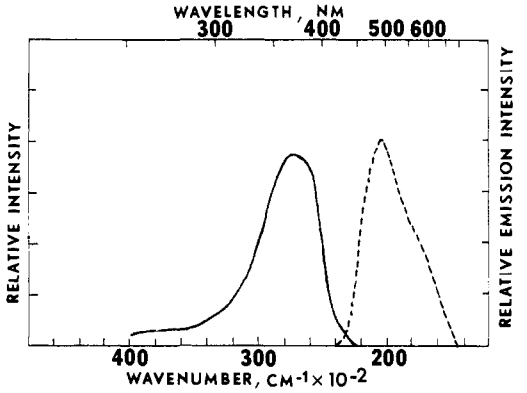

Figure 1. The corrected absorption (——) and emission (- - - -) spectra of *all-trans-n*-butyl Schiff base in 3MP at 77°K.

Relative quantum yields of fluorescence were calculated using 9,10-diphenylanthracene as a reference ($\phi_F = 1.0$),[13] using the method that we have previously described.[1,14] Five independent measurements of ϕ_F (0.07) of the *all-trans-n*-butyl Schiff base gave an average deviation of ±0.018 and a maximum deviation of ±0.03. These differences appear to be caused by differences in the path lengths and quality of the optical cells and dewars along with the method of correcting the observed emission for the phototube response, *i.e.*, a point by point correction or a center of mass correction.

The dependence of the fluorescence quantum yield on the excitation wavelength was calculated from the fluorescence excitation spectrum of the sample. Since the peak shape and maxima did not change as a function of the exciting wavelength, the intensity of the excitation spectrum at a given wavelength is then a measure of the relative emission intensity. This intensity is then corrected for the variations in the amount of light absorbed by the sample and for the lamp intensity at the different wavelengths. The intensity of the exciting lamp, $I(\lambda)$, is derived from a fluorescence excitation spectrum of Rhodamine B in ethylene glycol at room temperature.[15] The fluorescent quantum yield of Rhodamine B has been determined to be independent of the exciting wavelength, ±5% in the 280–530-nm region.[16]

Results and Discussion

We have observed emission from *all-trans*-, 9-*cis*-, and 13-*cis*-retinal, their corresponding *n*-butyl Schiff bases, the methyl Schiff base of *all-trans*-retinal, and the protonated methyl and *n*-butyl Schiff bases of *all-trans*-retinal at 77°K in 3-methylpentane. Emission has not been observed from 11-*cis*-retinal ($\phi_F < 0.001$);[1,2] however, we have observed an emission from the 11-*cis-n*-butyl Schiff base at 77°K.[17] No fluorescence has been observed from any of the foregoing solutions at room temperature, $\phi_F < 0.001$. The wavelengths of the absorption and emission maxima of these compounds are given in Table I along with their maximum fluorescence quantum yield and the exciting wavelength at which ϕ_F is at its maximum value. Figure 1 shows the corrected absorption and emission spectra of *all-trans*-retinylidene-*n*-butylamine at low temperature (77°K). Emission spectra of the 9-*cis*- and 13-*cis-n*-butyl Schiff bases are similar to that of the *all-trans-n*-butyl Schiff base. Absorption spectra of the isomeric *n*-butyl Schiff bases[18] as well as the absorption[19] and emission[1] spectra of the isomeric retinals are available.

There is very poor structure in the first observed band of the absorption spectrum as well as for the emission spectrum of both retinals and Schiff bases (see Figure 1 and ref 1, 18, 19). The band shapes are characteristic of a Franck–Condon forbidden type. The postion of the fluorescence band maxima of the retinals and Schiff bases is insensitive (±2 nm) to variations in the energy of excitation in the 300–440-nm region; however, the relative quantum yields of fluorescence of the retinals are quite sensitive to the exciting wavelength in the low energy absorption region.[1,9] The wavelength dependence of the quantum yield of fluorescence of *all-trans*-, 9-*cis*-, and 13-*cis*-retinal is similar. At longer wavelengths, the fluorescence quantum yield (ϕ_F) is at its highest value, decreasing steadily to about 390 nm. Throughout most of the first absorption band, from ~400 to ~300 nm, the quantum yield is constant within 25%. For *all-trans*-retinal, the maximum quantum yield of fluorescence is not quite 5%

(13) (a) E. J. Bowen and J. Sahu, *J. Phys. Chem.*, **63**, 4 (1959); (b) W. H. Melhuish, *J. Opt. Soc. Amer.*, **54**, 183 (1964); (c) I. B. Berlman, "Handbook of Fluorescence Spectra of Aromatic Molecules," Academic Press, New York, N. Y., 1965, p 130; (d) E. C. Lim, J. D. Laposa, and T. M. H. Yu, *J. Mol. Spectrosc.*, **19**, 412 (1966); (e) R. Li and E. C. Lim, *J. Chem. Phys.*, **57**, 605 (1972).

(14) D. E. Balke and R. S. Becker, *J. Amer. Chem. Soc.*, 90, 6710 (1968).

(15) Rhodamine B is run at room temperature in ethylene glycol while all of our samples, including 9,10-diphenylanthracene, are run at 77°K in 3MP. At 77°K, the light must pass through two parallel quartz windows and the liquid nitrogen coolant; this is not the case for the Rhodamine B excitation. To the same extent that the assumption is valid that the solvent and temperature changes of the two systems are negligible, then our calculation of the wavelength dependence of the quantum yield on the exciting energy is valid.

(16) W. H. Melhuish, *J. Opt. Soc. Amer.*, **52**, 1256 (1962).

(17) We have observed an emission from the 11-*cis-n*-butyl Schiff base at 77°K; however, it had two maxima (490 and 560 nm) one of which is characteristic of the all-trans Schiff base (490 nm). After room temperature irradiation of this sample, and reexamination of the emission spectrum at 77°K, the band narrowed and maximized at 490 nm. Consequently, the 11-cis Schiff base has an emission maximum at 560 nm in 3-methylpentane.

(18) W. H. Waddell, Ph.D. Dissertation, University of Houston, 1973.

(19) R. S. Becker, K. Inuzuka, and D. E. Balke, *J. Amer. Chem. Soc.*, **93**, 38 (1971).

at 440 nm.[20] Our determination of ϕ_F at 440 nm was done in two different manners: (1) three independent measurements of different samples gave an average value of $\phi_F = 0.045 \pm 0.005$ (compared to 9,10-diphenylanthracene, Table I); (2) determination of ϕ_F at 380 nm for two samples and generation of ϕ_F at 440 nm from the fluorescence excitation spectra (*vide supra*). The values in Table II

Table II. Calculated Dependence of ϕ_F upon the Exciting Wavelength[a]

Exciting wavelength, nm	ϕ_F retinals[b]			ϕ_F Schiff bases[b]		
	All-trans	9-cis	13-cis	All-trans	9-cis	13-cis
300	0.005	0.009	0.02	0.04_3	0.01_3	0.02_4
320	0.006	0.01	0.02_5	0.04_9	0.01_7	0.03_5
340	0.005	0.009	0.02_4	0.06	0.01_9	0.04
360	0.005	0.009	0.02_4	0.07	0.01_9	0.04_2
380[c]	0.006	0.009	0.02_4	0.05_9	0.01_5	0.03_3
400	0.008	0.013	0.03_7	0.05_2	0.01_1	0.02_7
420	0.01_9	0.03	0.06_6	0.03[d]	0.009[d]	0.01[d]
440	0.04_4	0.02_7	0.12_3			

[a] All spectra recorded at 77°K in 3MP at a concentration of $\sim 10^{-4}$ *M*. [b] Calculated from the fluorescence excitation spectrum and relative to ϕ_F at 380 nm. [c] ϕ_F at this wavelength calculated relative to 9,10-diphenylanthracene for all compounds considered. [d] Because of low absorption at this wavelength, the error in determining ϕ_F may be larger than usual.

for *all-trans*-retinal are the average of the measurements from (2) above. Table II also summarizes the wavelength dependence of the 9-*cis*- and 13-*cis*-retinals and the *n*-butyl Schiff bases of these three retinal isomers.

We have repeated the experiments on *all-trans*-retinal concerning the temperature dependence of the emission and have observed the same qualitative results that were previously observed.[1] We can observe emission from *all-trans*-retinal at ~100°K where the solution is fluid; however, the quantum yield is less than 25% of that value observed at 77°K, where the solution is rigid, exciting at the same wavelength. Warming this solution to room temperature results in the loss of the observed emission. Recall that we observe no emission from any of the retinals or Schiff bases ($\phi_F < 0.001$) at room temperature; however, a weak fluorescence has been observed from *all-trans*-retinal at room temperature using photon-counting techniques.[21] It has also been reported that emission was observed at room temperature using conventional techniques,[22] although this has not been confirmed by our work. The latter report[22] has included a preliminary estimate of the quantum yield as 0.01.

The dependence of ϕ_F upon the exciting wavelength for the Schiff bases of retinal exhibits a different behavior from the retinals themselves. Exciting in the low energy absorption region does not result in an increase in ϕ_F for the Schiff bases. The variation of the quantum yield with exciting wavelength is nearly 50% in the 300–400-nm region. For all three Schiff base isomers, the maximum quantum yield of fluorescence

(20) The previous determination of $\phi_F = 0.71$ at 440 nm for *all-trans*-retinal (see ref 1) has been found to be in error. Upon analysis of the data, we have found an operator error. The band maximum and shape are identical.

(21) B. E. Kohler, private communication, 1973.

(22) E. W. Abrahamson and S. M. Japar, "Handbook of Sensory Physiology," Springer-Verlag, Berlin, Germany, 1972.

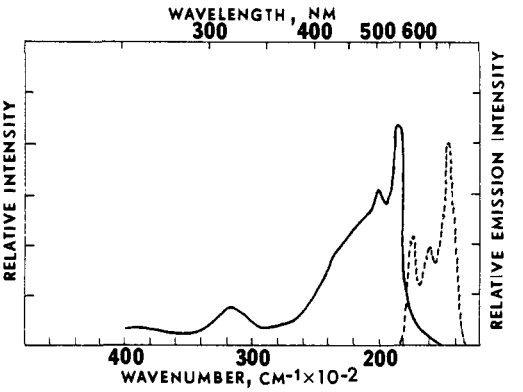

Figure 2. The corrected absorption (——) and emission (- - - -) spectra of *all-trans-n*-butyl Schiff base + HCl gas in 3MP at 77°K.

occurs near the absorption maxima; see Table I. Recall that this is in marked contrast to the retinals where the quantum yield is at its highest value at the absorption onset. We have also examined the monomethyl Schiff base of *all-trans*-retinal and observe emission with almost identical characteristics as those of the *n*-butyl Schiff base. It should be pointed out that the absorption spectra also have only slight differences.[18] Due to the possibility of having relatively large errors in the lamp curve (*vide supra*), it may be that in fact in the 300–400-nm region there is a negligible wavelength dependence of the fluorescence quantum yield for either the retinals or Schiff bases; however, the dependence of the quantum yield on exciting wavelengths near the low energy absorption onset in the retinals (400–440 nm) is real. For *all-trans*-, 13-*cis*-, and 9-*cis*-retinal, ϕ_F is nearly nine, six, and three times greater, respectively, at the low energy absorption onset (~440 nm) than near its maxima (~380 nm).

After addition of anhydrous hydrogen chloride gas to a 10^{-4} *M* solution of the methyl or *n*-butyl *all-trans* Schiff base, red shifts are observed in both the absorption and emission spectra recorded at 77°K. While the absorption maximum shifts from ~360 to ~460 nm (a shift of 6040 cm^{-1}), emission maximum red shifts from 490 to 680 nm (a shift of 5700 cm^{-1}) after protonation of the imine nitrogen. The shapes of the first absorption band and emission band are broad and unstructured, characteristic of a Franck–Condon forbidden type. The quantum yield of fluorescence is 0.005 (compared to 0.07 for the Schiff base) when exciting into the absorption maxima of the protonated Schiff base. After addition of anhydrous HCl gas to the *all-trans-n*-butyl Schiff base and subsequent generation of the protonated species that has transitions with maxima at 542 and 318 nm,[11] a structured emission could be detected at 77°K with bands at 575, 625, and 685 nm; see Figure 2. The band shapes of the absorption are characteristic of a Franck–Condon allowed type. Identical emission results can be obtained when exciting at 315, 500, or 542 nm. The identical nature of the fluorescence excitation spectra obtained by monitoring at 575, 625, and 680 nm allowed us to conclude that the three emission bands arose from the same emitting species. In fact, the transition near 320 nm was readily detected in the excitation spectra recorded at the three wavelengths since there was little if any dependence of the quantum

yield upon the exciting wavelength. This latter behavior is quite similar to the Schiff bases; *vide supra*. ϕ_F was calculated to be 0.03, but may in fact be larger since there is appreciable overlap of the onsets of absorption and emission (Figure 2), and self-absorption of part of the emission may occur. This latter process would greatly affect the height and position of the first band in emission (575 nm). We have estimated that after correcting for self-absorption the intensity of the first emission band (575 nm) may be double since at 575 nm ~50% of the light is reabsorbed by the protonated Schiff base. The height and position of the third emission band (~680 nm) are considerably altered by the phototube correction.

We were unable to detect any phosphorescence for any of the molecules studied to 820 nm. Based on energy transfer experiments,[23] the 0–0 band for phosphorescence of *all-trans*-retinal has been estimated to be at 880 nm. More recent data from the direct singlet–triplet absorption spectrum of *all-trans*-retinal[24] have assigned the 0–0 of the singlet–triplet transition to be at 803 nm, the lowest energy band that they have observed. Using photon-counting techniques, recent attempts to observe phosphorescence from *all-trans*-retinal to ~1050 nm were also unsuccessful.[25] This was unexpected since a recent redetermination of the intersystem crossing quantum yield (ϕ_{ISC}) for *all-trans*-retinal at room temperature found the value to be near 0.60 ± 0.10[26] as opposed to the early estimates of 0.11 at room temperature[27] and 0.28 at −73°.[28] The quantum yield of photoisomerization (ϕ_{PI}) at −65° was low, ~0.003,[29] and thereby would be essentially zero at −196°C (77°K). If indeed phosphorescence is absent, it is evident that there are efficient internal conversion modes from the lowest triplet state (also see later discussion).

In addition to the reevaluation of the intersystem crossing quantum yield, the quantum efficiency for photoisomerization from the triplet state ($^3\phi_{PI}$) of *all-trans*-retinal has been determined to be 0.17 at room temperature[30] based on energy transfer to the triplet state. The contribution of photoisomerization from the triplet state to ϕ_{PI} is then $(\phi_{ISC})(^3\phi_{PI})$ or about 0.1. This number falls within the range that had been previously reported for the total photoisomerization quantum efficiency[29] for *all-trans*-retinal at room temperature (maximum value of 0.2 and minimum value of 0.06). However, recent measurements have determined that the total quantum efficiency for photoisomerization is 0.04.[21]

We know that

$$\phi_{Total} = \phi_F + {}^1\phi_{PI} + \phi_{ISC} + {}^1\phi_{IC} + {}^1\phi_{PC}$$

where $^1\phi_{IC}$ is the quantum yield of internal conversion to the ground state from an excited singlet state. For *all-trans*-retinal at room temperature, ϕ_F is essentially zero (<0.001), $\phi_{ISC} = 0.6 \pm 0.1$, ϕ_{PI} ranges from 0.2 to 0.04, and $^3\phi_{PI} = 0.1$, and we can substitute these values in eq 1 and estimate $^1\phi_{IC}$. Neglecting the possibility that thermally reversible photochemistry occurs from a singlet state, the minimum value of $^1\phi_{IC}$ is

$$^1\phi_{IC} = 1.0 - \phi_{ISC}(max) - {}^1\phi_{PI}(max) - \phi_F(max)$$
$$= 1.0 - 0.7 - 0.1 - 0 = 0.2$$

where $^1\phi_{PI} = \phi_{PI} - {}^3\phi_{PI}$. The maximum value of $^1\phi_{IC}$ is 0.5 since ϕ_{ISC} has a minimum value of 0.5 and all photoisomerization would then be postulated to occur from the triplet state. Furthermore, of the quanta that reach the triplet state (0.6 ± 0.1) only 0.1 result in photoisomerization; therefore, 0.5 ± 0.1 are internally converted from the triplet state. The total amount of internal conversion could be as high as 0.96 or as low as 0.8 (assuming an upper limit of 0.2 for the maximum quantum yield of photoisomerization).

At 77°K the photoisomerization of *all-trans*-retinal is essentially absent, a fluorescence does occur, ϕ_F(max) = 0.05, and since no phosphorence has been observed thus far, ϕ_P can be considered to be zero. Based on the foregoing premise, 0.95 of the total quanta would be internally converted. However, this analysis neglects the photochemistry we have previously observed at 77°K[1,2] because no quantum yield data are available.

At room temperature or 77°K, no emission has been observed from 11-*cis*-retinal[1,2] ($\phi_F < 0.001$), but the quantum yield of photoisomerization is reported to be 0.2 at room temperature.[29] The efficiency of photoisomerization from the triplet state[30] has been determined to be 0.75; however, it is unfortunate that no recent determination of ϕ_{ISC} has been made for this isomer. A previous measurement[28] estimated ϕ_{ISC} to be near (within 15%) that of *all-trans*-retinal. If indeed ϕ_{ISC} were also to be near 0.6 for 11-*cis*-retinal, then there would be more photoisomerization out of the triplet state (0.45) than has been measured in total (0.2 at room temperature). A redetermination of ϕ_{PI} and ϕ_{ISC} for 11-*cis*-retinal would certainly help to resolve this problem.

No triplet–triplet absorption[28] has been observed for the *n*-propyl Schiff base of *all-trans*-retinal or its protonated form nor has phosphorescence been observed. No measurement of ϕ_{PI} for the retinal Schiff bases or protonated Schiff bases has been made. Because of these facts, we can say little about the fate of the absorbed quanta until further information is obtained (in addition to our fluorescence quantum yield data).

One of the most difficult problems to resolve is the marked wavelength dependence for ϕ_F at the long wavelength absorption onset in the retinals which is not present in the Schiff bases (Table II). Also, *all-trans*-retinal shows a significant amount of intersystem crossing (*vide supra*), whereas its Schiff base does not.[28] Before considering some possible explanations for these differences, some discussion of the state ordering in these and similar molecules is necessary. Of first importance is to establish the basis for expecting a low lying $^1(n,\pi^*)$ state to exist in the retinals, that is, near the lowest strongly allowed $^1(\pi,\pi^*)$ state (^1B_u for C_{2h} symmetry).

An experimental study of the polyene series $CH_3(HC{=}CH)_nCHO$ indicates that there is a merging of a weak long wavelength transition, $*\pi \leftarrow n$, with an adja-

(23) A. V. Guzzo and G. L. Pool, *J. Phys. Chem.*, **73**, 2512 (1969).
(24) R. A. Raubach and A. V. Guzzo, *J. Phys. Chem.*, **75**, 983 (1971).
(25) A. M. Schaffer and J. Callis, unpublished.
(26) R. Benasson, E. J. Land, and T. G. Truscott, *Photochem. Photobiol.*, **17**, 53 (1973).
(27) W. Dawson and E. W. Abrahamson, *J. Phys. Chem.*, **66**, 2542 (1962).
(28) K. H. Grellmann, R. Memming, and R. Livingston, *J. Amer. Chem. Soc.*, **84**, 546 (1962).
(29) A. Kropf and R. Hubbard, *Photochem. Photobiol.*, **12**, 249 (1970).
(30) R. A. Raubach and A. V. Guzzo, *J. Phys. Chem.*, 77, 889 (1973).

cent strong transition $^*\pi \leftarrow \pi$ (^1B_u) as n increases.[31] The data indicate that by the time $n = 4$ or 5 the two transitions are essentially degenerate. We have carried out calculations on a series of polyenes of the type $H(HC{=}CH)_nCHO$ to determine the energy of n,π^* and π,π^* singlet and triplet states.[32] At $n = 1$, the lowest $^1(\pi,\pi^*)$ state (^1B_u) is at considerably higher energy than the $^1(n,\pi^*)$ state. As n increases the ^1B_u state decreases rapidly in energy while the n,π^* state energy undergoes almost no change. At $n = 4$ the states are essentially degenerate. Although retinal has $n = 5$, substantial twisting about the 6–7 single bond to the ring[33] reduces the effective value of n to between 4 and 5. Both the experimental and theoretical results give strong evidence that the lowest strongly allowed $^1(\pi,\pi^*)$ state (^1B_u) and the lowest $^1(n,\pi^*)$ state are very close in energy. In addition, there is also the complication of another $^1(\pi,\pi^*)$ state ($^1A_g^-$) being close in energy to the ^1B_u state in polyenes[34–36] and therefore could be close to the $^1(n,\pi^*)$ in retinals. It is clear that all three of the states $^1(n,\pi^*)$, $^1A_g^-$ (π,π^*), and ^1B_u (π,π^*) can be energetically close. It appears that it will be difficult if not impossible to determine the actual state ordering and indeed the states are probably highly mixed.

In the case of Schiff bases no $^1(n,\pi^*)$ is expected near the low lying π,π^* singlets. This is based on both absorption spectral data[37] and the lack of triplet–triplet absorption for the Schiff base[28] of *all-trans*-retinal. Of course, no low lying $^1(n,\pi^*)$ state would be expected for the protonated Schiff bases.[38]

The manner in which the presence of a low lying $^1(n,\pi^*)$ state could affect the dependence of ϕ_F upon wavelength could be one of two ways:[39] (1) cause a wavelength dependent photochemistry in retinals not possible in the Schiff bases; (2) cause a wavelength dependence for intersystem crossing.

Photochemistry does occur for the retinals at 77°K[1,2] which we have not observed for the Schiff bases. This could very well be related to the differences in photochemical properties between carbonyl and imino groups. In general, photochemistry can be wavelength dependent, and thereby result in altering ϕ_F as a function of wavelength.[40,41] In the retinals, this could arise because of excitation of (1) different states particularly one of a different configuration as $^1(n,\pi^*)$ *vs.* $^1(\pi,\pi^*)$, or (2) vibronic levels within states. These explanations are parallel to ones we have given previously,[1,2] although the exact nature of the different states was not identified. In addition, although photoisomerization is not expected for the *all-trans*-retinal at 77°K, it does occur for the cis isomers. The photoisomerization quantum yield is in general wavelength dependent[29] for the retinals; however, no studies have been made near the absorption onset where the greatest change in ϕ_F is observed.

Because of the presence of a low lying $^1(n,\pi^*)$ state for retinals, direct first order spin–orbit coupling could occur to a $^3(\pi,\pi^*)$ or spin–orbit coupling could occur by a second order process involving vibronic mixing of π,π^* and n,π^* states of the same multiplicity. Theoretically, at least, the degree of intersystem crossing could change as a function of the state or vibronic level excited and result in a wavelength dependence for ϕ_F. However, in all known cases internal conversion between states or within a state competes efficiently with intersystem crossing so that fluorescence and intersystem crossing occur from a thermalized set of vibronic levels in the first electronic singlet state.[40,42] Moreover, the expected small energy difference between the three lowest singlets in retinals would tend to increase the rate of internal conversion.

Because of an uncertainty in knowing the correct state order and an uncertainty in relating an increase or decrease in ϕ_F to any one state, any discussion specifically relating the wavelength at which the maximum of ϕ_F occurs with a particular state is precarious. Nonetheless, we believe that presence of a low lying $^1(n,\pi^*)$ in the retinals could cause a wavelength dependent photochemical process not permitted for the Schiff bases (*vida supra*). If we make the reasonable assumption that this competing photochemical process is more likely to occur in the $^1(n,\pi^*)$ state compared to a $^1(\pi,\pi^*)$ state, then the wavelength associated with the maximum of ϕ_F does not correspond to the location of the $^1(n,\pi^*)$.[43]

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(31) E. R. Blout and M. Fields, *J. Amer. Chem. Soc.*, 70, 189 (1948).

(32) K. Inuzuka and R. S. Becker, unpublished. All calculations were of the SCF-MO-CI type using Pariser-Parr approximation for the repulsion integrals. The nonbonding orbital energy was calculated in a unique way as has been described by us for some other aldehydes: K. Inuzuka and R. S. Becker, *Bull. Chem. Soc. Jap.*, **45**, 1557 (1972).

(33) R. D. Gilardi, I. L. Karle, and J. Karle, *Acta Crystallogr., Sect. B*, **28**, 2605 (1972), and references therein.

(34) B. S. Hudson and B. E. Kohler, *Chem. Phys. Lett.*, **14**, 299 (1972).

(35) R. L. Christensen and B. E. Kohler, *Photochem. Photobiol.*, **18**, 293 (1973).

(36) K. Schulten and M. Karplus, *Chem. Phys. Lett.*, **14**, 305 (1972).

(37) J. R. Platt, "Radiation Biology," Vol. 3, D. Davey, Inc., Hartford, Conn., 1956, p 92.

(38) No triplet–triplet absorption spectrum has been observed for two different protonated Schiff bases of *all-trans*-retinal (see ref 27 and 28).

(39) It should be noted that the anomalous excitation spectrum of *all-trans*-retinal has been explained on the basis of dimer formation: T. A. Moore and P. S. Song, *Nature (London), New Biol.*, **243**, 30 (1973). From a determination of the excitation spectra at several different fluorescence wavelengths, a determination of the fluorescence spectra as a function of exciting wavelength, and a determination of ϕ_F as a function of retinal concentration, we conclude that the anomalous excitation spectra of retinals cannot be explained on the basis of dimer formation: W. H. Waddell, A. M. Schaffer, and R. S. Becker, to be published.

(40) R. S. Becker, E. Dolan, and D. E. Balke, *J. Chem. Phys.*, **50**, 239 (1969).

(41) N. W. Tyer and R. S. Becker, *J. Amer. Chem. Soc.*, **92**, 1295 (1970).

(42) J. B. Birks, "Photophysics of Aromatic Molecules," Wiley-Interscience, London, 1970.

(43) The location of the maximum in the excitation spectrum of *all-trans*-retinal in dried 3MP *vs.* a hydroxylic solvent is the same (±2 nm). This supports our contention that the wavelength associated with the maximum of ϕ_F does not correspond to the location of the $^1(n,\pi^*)$ state.